

Biochemistry 2018: S-nitrosylation in apoplastic fractions of *Brassica juncea* hints regulation of wide spectrum of stress/ redox/metabolic pathways and spatial distribution of targets in cold stress

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Abstract

Apoplast or the extracellular space is an important site for stress signal perception or initial signaling in response to environmental stimuli. Nitric oxide (NO) is a signaling molecule which modify proteins by S-nitrosylation (a posttranslational modification), resulting in the formation of S-nitrosothiols. However, NO mediated signaling in the apoplast remains largely unknown. To decipher this, apoplastic proteins were extracted using vacuum infiltration. Western blotting using anti-RuBisCO antibody and glyceraldehyde 6-phosphate dehydrogenase activity assay, showed purity of the extracts without any chloroplastic and cytosolic contamination. NO was measured in the apoplast of *Brassica juncea* seedlings using iNO measuring system, which showed that non-enzymatic nitrite reduction was increasing NO production during cold stress. Thiol pool quantification using Ellman's assay showed cold-induced increase in the protein (including S-nitrosothiols) as well as non-protein thiols. About 52 S-nitrosylated spots were resolved using Biotin Switch Technique, neutravidin agarose chromatography and 2-DE in cold stress. Moreover, identification of cold-stress-modulated putative S-nitrosylated proteins by nLC-MS/MS showed that only 38.4% targets with increased S-nitrosylation were secreted by classical pathway, while the majority (61.6%) of these was secreted by unknown/non-classical pathways. About 41% and 38% targets were metabolic/cellwall-modifying and stress-related, respectively, suggesting the potential role(s) of S-nitrosylation in regulating these responses. S-nitrosylation increased glutathione S-transferase and dehydroascorbic reductase activity, promoted ROS detoxification by ascorbate regeneration and hydrogen peroxide detoxification in cold-stress. A total of 48 putative S-nitrosylated targets were identified, including 25 novels. This study was the first attempt which showed NO mediated cold-stress signaling in the apoplast..

This work is partly presented at International Conference on Biochemistry, Proteomics & Bioinformatics May 16-17, 2018 Singapore